2. REVIEW OF LITERATURE

The increased use of various types of pesticides in agriculture has led to much greater emphasis on the possibility of serious environmental contamination arising from their use. The presence of pesticides in the environment is attributable to their application into soil or their use as spray on plants. Presence of pesticide and their degradation products in the environment is inevitable as only a small percentage of total quantity of pesticide used is actually involved in the control of pests, and diseases. The excess of residue may be removed by physical, chemical or biological means. The success of such methods depends on the type of pesticide and the environmental conditions. The most effective mechanism of environmental decontamination of pesticides is biological. Microorganisms which populate much of the earth's surface are excellent biological incinerators (Alexander, 1969). Sometimes a compound may persist in environment because it is not used as carbon source by microorganisms and such a chemical is termed recalcitrant. Some members of chlorinated hydrocarbon insecticides have this property (Alexander, 1967).

While pesticides are usually aimed at target organisms within specific components of the environment, the methods of application and the subsequent influences on these chemicals often result in a more widespread distribution. Thus, chemical structure and consequently biological activity may be altered by physical, chemical and biological
agencies. Hence, most pesticides which are designed for specific uses, but their specificity is rarely absolute. Harmful effects may therefore be exerted on some non-target organisms in soil. Such effects may not only be caused by the pesticide but also by any transformation products and may result in the elimination, decrease or modification of microbial transformations such as nitrification, ammonification and organic matter decomposition which are essential for soil fertility and agricultural productivity (Alexander, 1961; Burges and Raw, 1967). Population of microorganisms may also adapt to the presence of pesticide by change in species diversity or by adaptation of enzyme systems, so that pesticide may be more rapidly metabolized and consequently eliminated from the environment (Audus, 1964; Kearney and Kaufman, 1965). Therefore, the study of interaction between pesticides and soil microorganisms is clearly of great importance and has been stressed by various authors (Bollen, 1961; Kreutzer, 1963; Audus, 1964; Alexander, 1969; Cullimore, 1971; Kaufman, 1974), so that as the newer pesticide become available these may be judiciously used for pest and disease control without harming the useful microorganisms or their activities in soil.

2.1. Chemistry of Dithiocarbamates:

All the dithiocarbamate fungicides available commercially may be regarded as derivatives of carbonic acid (1), in which an hydroxyl group is replaced by an amino group to give carbamic acid (2), the replacement of
two oxygen atoms by sulfur gives the dithiocarbamic acid (3).

\[
\begin{align*}
\text{HO} & \quad \text{HO} \\
C &= 0 & C &= 0 \\
\text{HS} & \quad \text{C = S}
\end{align*}
\]

(1) (2) (3)

The dithiocarbamic acid is unstable and the fungicidal derivatives of this compound can be classified into three groups: Metallic Dithiocarbamates (4) e.g., ziram and ferbam; thiuram disulphides (5) e.g., thiram, and bisdithiocarbamates (6) e.g., nabam, maneb and zineb (Nene and Thapliyal, 1979).

\[
\begin{align*}
R \quad N & \quad S \\
C \quad -S&-\text{metal} \\
(4) & \\
R \quad N & \quad S \\
C \quad S-S-C-N & \quad \text{R} \\
(5) & \\
H & \quad N \quad S \\
C \quad -S &-\text{metal} \\
(6) & \\
H & \quad N \quad S \\
C \quad -S &-\text{metal}
\end{align*}
\]

The structure and properties of commercially available dithiocarbamates i.e., ziram, ferbam, thiram, nabam, maneb and zineb are given in Table 1 (Melnikov, 1971; Nene and Thapliyal, 1979).

2.2. Development of Dithiocarbamates:

The development of purely organic fungicides began with the discovery of the fungicidal activity of dithiocarbamates which had been originally developed as vulcanizing agents for the rubber industry. The dithiocarbamates and their derivatives are one of the most important groups of organic fungicides for controlling plant diseases (Cremlyn, 1978).
<table>
<thead>
<tr>
<th>Compound</th>
<th>Solubility</th>
<th>Melting Point</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ziram (Zinc dimethyl dithiocarbamate)</td>
<td>Insoluble in water, soluble in ethanol, soluble in chloroform</td>
<td>246°C</td>
<td>326.4</td>
</tr>
<tr>
<td>Ferbam (Ferrous dimethyl dithiocarbamate)</td>
<td>Insoluble in water</td>
<td>416.5°C</td>
<td>416.5</td>
</tr>
<tr>
<td>Thiram (Tetramethylthiuram disulphide)</td>
<td>Insoluble in ethanol, soluble in chloroform</td>
<td>165-175°C</td>
<td>240.4</td>
</tr>
</tbody>
</table>

* 65 ppm in water, insoluble in ethanol, soluble in chloroform.
* Decomposes 120 ppm in water, insoluble in ethanol, soluble in chloroform.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular weight</th>
<th>Melting point</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>4. Maneb (Manganous ethylenebisdithiocarbamate)</td>
<td>265.3</td>
<td>Decomposes</td>
<td>Insoluble in water and organic solvents.</td>
</tr>
<tr>
<td>5. Zineb (Zinc ethylenebisdithiocarbamate)</td>
<td>275.5</td>
<td>Decomposes</td>
<td>Insoluble in water and organic solvents.</td>
</tr>
<tr>
<td>6. Nabam (Disodium ethylenebisdithiocarbamate)</td>
<td>256.3</td>
<td>Decomposes</td>
<td>Greater than 20% in water, insoluble in organic solvents.</td>
</tr>
</tbody>
</table>
Dithiocarbamate fungicides were introduced in the field of pest control by Tisdale and Williams (1934). Hester (1943) produced disodium ethylene bisdithiocarbamate. This compound was shown to possess outstanding fungitoxic properties (Diamond et al., 1943) but was unstable. Heuberger and Manns (1943) discovered the stabilizing influence of zinc sulphate and lime and resulted in the development of zineb and maneb as field fungicides. The details of history and development of dithiocarbamates as field fungicides and their role in opening the organic era has been given by Horsfall (1956) and Martin (1959). Metcalf (1971) discovered the fungicidal activity of ziram and ferbam. Gupta et al. (1974) studied the synthesis and insecticidal activity of several dithiocarbamates. A vast number of mixed formulations and combinations of maneb and zineb with other fungicides have been introduced (Nene and Thapliyal, 1979).

2.3. Mechanism of Action:

The uses of dithiocarbamates and their derivative as fungicides have been reviewed and their mode of fungicidal action has been the subject of considerable study (Rich and Horsfall, 1961; Thorne and Ludwig, 1962; Torgenson, 1969; Metcalf, 1971). There are some differences in their fungicidal properties which suggest that the N,N-dimethyldithiocarbamates may have a different mode of action from that of the ethylenebisdithiocarbamates. Thus they possess a
distinctive spectrum of activity against various species of fungi (Cremlyn, 1978). Kaars Sijpesteijn and van der Kerk (1952) found that L-histidine would reverse the fungicidal activity of thiram, tetramethylthiuram monosulphide and sodium dimethyldithiocarbamate but not that of sodium ethylene bisdithiocarbamates. Halls (1969) has presented a review of dithiocarbamates in which comparison was made of the properties of mono and dialkyldithiocarbamates and their uses.

Dialkyldithiocarbamates (thiram, ziram and sodium dimethyldithiocarbamates) are derived from dialkylamines and are strong chelating agents. It was thought that they acted by depriving the cell of needed metal. However, it was indicated that a heavy metal ion was required for high toxicity of these compounds (Goksoyr, 1955; Kaars Sijpesteijn et al., 1957; Richardson and Thorne, 1961). Interaction of copper ions and dimethyldithiocarbamate (DDC ion) result in the formation of two complexes viz., the unsaturated positively charged 1:1 complex and the saturated uncharged 1:2 complex. Goksoyr (1955) observed the toxicity of dialkyldithiocarbamates at low concentration due to 1:1 complex. This 1:1 (DDC : Cu)\(^+\) complex probably bind to metal components of cell and prevents growth.

Lowe and Phillips (1962) suggested that mode of action of dialkyldithiocarbamates might be associated with the incorporation of copper into porphyrin precursor of an essential haem type pigment. Owens and Rubinstein (1964) suggested that toxicity of dialkyldithiocarbamates to be
due to the free radicals by virtue of their reaction with sulphydryl group essential for the functioning of the sulphydryl enzymes. Bates and Tweedy (1968) reported that thiram had no effect upon glycolysis but inhibited the activity of pentose pathway and acetate metabolism in *Fusarium oxysporum*. Thus the mechanism of action of dialkyl-dithiocarbamates in fungal cells still remains obscure (Nene and Thapliyal, 1979).

Monoalkyl dithiocarbamates (nabam, maneb and zineb) which are derived from monoalkylamines act by different mechanism than the dialkyl dithiocarbamates. Ludwig and Thorne (1953) found ethylenethiuram monosulphide (ETM) among the decomposition products of nabam and thought that ETM might account for nabam toxicity. Kaars Sijpesteijn and van der Kark (1954) however, postulated that Ethylene thiuram monosulfide (ETM) was formed via an isothiocyanate intermediate and that the two compounds were in equilibrium. Hassal (1969) found pure ethylenebis dithiocarbamates inactive before their exposure to air. He also observed that isothiocyanates acted by virtue of their reaction with thiol compounds in fungal cell. Lukens (1971) suggested that the mechanism of action probably involved the oxidative decomposition of these bisdithiocarbamates into products as thiram, carbon disulphide and possibly ethylene diisothiocyanate. Formation of volatile methylisothiocyanates from vapam further supports the isothiocyanate theory (Nene and Thapliyal, 1979).
However, there is evidence to suggest that isothiocyanates might not be entirely responsible for toxicity of monoalkyldithiocarbamates. Wedding and Kendrick (1959) found different toxicological effects between N-methyl-dithiocarbamate and methylisothiocyanate. N-methyl-dithiocarbamate, but not methylisothiocyanate, caused alteration in the permeability of the mycelium of *Rhizoctonia solani*.

2.4. Effect of Dithiocarbamates on Soil Biological Transformations:

The extensive use of agricultural pesticides has made it essential to see the possible effects of these chemicals on microbiological activities affecting soil fertility, plant nutrition and occurrence of plant diseases. Microorganisms play an important role in soil fertility by chemical (makes nutrients available for plants), physical (maintain the soil structure) and biological (antagonize the plant pathogen) means. Anderson (1978) made an attempt to obtain an overall summary of the general effects that a particular group of pesticides might have on certain soil microbiological processes (Table 2). Mitterer et al. (1981) studied the effect of several fungicides on soil biological activity which were found to affect both CO₂ release and enzyme activity influencing straw decomposition and other metabolic activities.

Fungitoxic levels of metallic dithiocarbamates suppress nitrification in soil (Jaques *et al.*, 1959; Chandra and Bollen, 1961). Thiram was found to decrease soil
Table 2: Summary of pesticide effect ratio* on microbial processes in soil.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Herbicide</th>
<th>Fungicide</th>
<th>Insecticide</th>
<th>Other pesticides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial number</td>
<td>1.20</td>
<td>3.50</td>
<td>1.30</td>
<td>1.00</td>
</tr>
<tr>
<td>Nitrification</td>
<td>1.40</td>
<td>0.54</td>
<td>0.82</td>
<td>0.32</td>
</tr>
<tr>
<td>Denitrification</td>
<td>1.82</td>
<td>i.d.a.</td>
<td>i.d.a.</td>
<td>i.d.a.</td>
</tr>
<tr>
<td>Rhizobia and legume nodulation</td>
<td>0.94</td>
<td>1.00</td>
<td>0.78</td>
<td>i.d.a.</td>
</tr>
<tr>
<td>Free living nitrogen fixation</td>
<td>1.65</td>
<td>i.d.a.</td>
<td>1.75</td>
<td>i.d.a.</td>
</tr>
<tr>
<td>Fungi and actinomycites</td>
<td>1.09</td>
<td>0.5</td>
<td>1.43</td>
<td>0.55</td>
</tr>
<tr>
<td>Pathogens and their antagonist</td>
<td>0.81</td>
<td>4.00</td>
<td>i.d.a.</td>
<td>i.d.a.</td>
</tr>
<tr>
<td>Algae</td>
<td>0.45</td>
<td>i.d.a.</td>
<td>i.d.a.</td>
<td>i.d.a.</td>
</tr>
<tr>
<td>Cellulolytic activity</td>
<td>1.31</td>
<td>i.d.a.</td>
<td>1.10</td>
<td>0.62</td>
</tr>
<tr>
<td>Respiratory activity</td>
<td>0.91</td>
<td>0.40</td>
<td>2.00</td>
<td>1.40</td>
</tr>
<tr>
<td>Other enzymic activity</td>
<td>1.70</td>
<td>0.44</td>
<td>2.00</td>
<td>0.66</td>
</tr>
<tr>
<td>Ammonification</td>
<td>1.74</td>
<td>1.30</td>
<td>1.84</td>
<td>1.20</td>
</tr>
</tbody>
</table>

* The ratio of positive to negative effects describes the effect ratio.

i.d.a. = insufficient data available.
respiration initially and depression in carbon dioxide production proportional to its concentration; subsequent stimulation in respiration was probably due to the utilization of decomposition products by microorganisms (Radwan, 1965). The effect of benomyl, maneb and dyrene on nitrogen transformations in soil was studied (Mazur and Hughes, 1975) and found that benomyl stimulated nitrogen mineralization, but both dyrene and maneb had inhibitory effect after four weeks of incubation.

Wainwright and Pugh (1975b) showed that high concentration (250 µg g⁻¹ soil) of benomyl and thiram lowered the concentration of aminoacid after 28 days while low concentration (50 µg g⁻¹ soil) resulted in marked increase in the amount of aminoacid nitrogen extracted. The inhibitory effect of hexachlorocyclohexane and carbofuran on nitrification in flooded soil was observed by Ray et al. (1980).

Application of pesticide may change the relative number of microorganism(s) which are antagonistic to others. Application of dimethylcarbamates (Moje et al., 1957; Domsch, 1970) stimulated the growth of Trichoderma. Increased number of this fungus drastically retarded the population of Armillaria, Phythium, Rhizoctonia and Phytophthora spp. and other soil borne pathogens (Alexander, 1969).

2.4.1. Effect of Dithiocarbamates on the Growth of Total and Specific Microorganisms:

Unless bacteria are directly inhibited by fungicide,
the effect of fungicides on them may be expected to be stimulatory in providing additional organic substrates (as killed fungi) for their growth and also where applicable by reducing antibiotic production by fungi. Decreased fungal activity might further reduce the competition for existence and nutrients, but might also decrease the availability of certain types of nutrients by inhibiting fungal degradation of organic materials, such as lignin (Anderson, 1978).

2.4.1.1. Effect on Bacterial Numbers:

Naumann (1971c) found that bacterial population decreased to 60% of that in untreated soil during the first week after treatment with captan (20 Kg ha$^{-1}$ loam soil) and then increased over the next three weeks to 180% of the control before finally returning to the control level. Thiram increased bacterial numbers steadily to 180% of the untreated soil population. Three soil bacterial species were used by Chinn (1973) to bioassay eight fungicides applied to soil. Methylmercury dicyandiamide (MMDD) was the only fungicide inhibiting bacteria at 1 ppm. MMDD and pyrazophos were the most inhibitory followed by thiram and maneb in decreasing order; captan showed little or no activity.

Houseworth and Tweedy (1973) showed that captan and thiram at 0.1 or 1.0 Kg ha$^{-1}$ in a silt loam soil, caused fluctuations in bacterial numbers which were inversely
related to the fungal population and in combination with atrazine neither fungicide exhibited synergistic or antagonistic effects on the population. In an unspecified soil type (pH 5.3 and 3.1 % organic matter), captan (9 Kg ha\(^{-1}\)) and thiram (6.7 or 13.4 Kg ha\(^{-1}\)) significantly increased number of heterophilic soil bacteria while benomyl (4 or 20 Kg ha\(^{-1}\)) had little effect (Wainwright and Pugh, 1974). Dyrene and maneb (1.5, 6.0, 24 and 96 Kg ha\(^{-1}\)) in acid lateritic clay (pH 5.0) and in alluvial loam (pH 7.7), increased bacterial population, but the effect dissipated ten months after treatments, except with the highest rate of maneb (Dubey and Rodriguez, 1974). Incubation of thiram with soil for two months stimulated bacterial population (Kecskes and Schmidt, 1976).

Pure culture of *Azotobacter chroococcum* was inhibited by zineb (5000 and 50,000 ppm), captan (3000 and 30,000 ppm), ferbam (30,000 ppm) while antibiotic mixture, fungicidin, had no effect at 5000 ppm (Langkramer, 1970). The sensitivity of rhizosphere isolates to thiram and its degradation product, sodium dimethyldithiocarbamate was studied at pH values of 5-9 (Sud and Gupta, 1972). *A. chroococcum* was more sensitive than other isolates, especially at pH 7, and isolates from roots of different plants varied in their susceptibility to these chemicals. Amylolytic, cellulolytic and NH\(_4\)-oxidizing bacteria were not significantly influenced by treatment with benomyl (2 Kg ha\(^{-1}\)) and had no effect on *Azotobacter* spp. in sandy soil (van Fassen, 1974).
2.4.1.2. Fungi and Actinomycetes:

Fungicides are designed to kill undesirable fungi but other fungi are also affected. Thiram caused a reduction in fungal species and the survivors were predominantly species of Trichoderma and Penicillium (Richardson, 1954). Vapam (60 to 500 ppm) stimulated actinomycetes and killed fungi and bacteria in the treated soil and nabam (60 to 400 ppm) inhibited fungi only of which eventually Trichoderma developed to dominate the population (Domsch, 1970). Thiram and ferbam at 31 and 125 ppm inhibited Botrytis cinerea in a pure culture. The growth of this fungus was also prevented by 15.6 ppm of neban, zineb and ziram (Parry and Wood, 1959). Thiram (1.2 and 2.5 Kg ha⁻¹) increased the frequency of reisolation of Rhizoctonia solani from soil seeded with this pathogen (Popov and Zdrozhevskaya, 1972). Agnihotri (1974) found that soil application of TMTD (375, 187.5 and 93.7 ppm) and seed treatment (0.1 %) effectively controlled damping off of chillies, caused by Pythium irregularae. Soil treatment was better than seed treatment. The seedling in thiram amended soil (93.7 ppm) were healthy with lush green foliage and developed good root system. Dithiocarbamate fungicides were found most active against Rhizopus sp. and TMTD markedly inhibited Mucor mucedo (Cohen and Dennis, 1975). Incubation of thiram with soil for two months inhibited actinomycetes and fungi (Kecskes and Schmidt, 1976). Oku et al. (1979) observed that under field condition heavy application of thiram reduced fungal
population to 1/6th by the day following treatment but normal level was returned on 6th day. Thiram (0.2, 0.1 and 0.05%) inhibited sporulation of *Myrothecium roseum* at all the concentrations used (Sohi and Kalra, 1982). Thiram (0.3%) when used as dry powder and in the form of slurry gave better effectiveness as compared to sulphur, in reducing fungal population associated with sorghum seeds (Raut and Wangikar, 1982).

Klopping and van der Kerk (1951) showed that respiration of *Aspergillus niger* and *Penicillium italicum* was reduced by sodium dimethyl and diethylcarbamates, but only a concentration in excess of those required to inhibit the growth of fungi. McCallan and Miller (1953) and McCallan *et al.* (1954) obtained similar results with ferbam on spores of *Neurospora sitophila*. Goksoy (1955) found that in baker's yeast cell acetate oxidation was more strongly inhibited than ethanol or glucose by dithiocarbamates. Acetyl CoA Kinase was not inhibited by sodium or zinc dimethyldithiocarbamate but was strongly inhibited by thiram. Similar results were obtained by Owens and Hayes (1964) in intact conidia of *Neurospora sitophila*. They found that ferbam and thiram inhibited the formation of citrate from acetate, whereas the zinc and copper salts had no discernible effect in this process at the minimum lethal dose. However, the zinc and copper salts caused accumulation of citrate. They found that ferbam inhibited citrate synthesis through interaction with CoA whereas the zinc and copper salts probably interact with aconitase, a Fe$^{2+}$ dependent enzyme,
thus preventing conversion of citrate beyond the step in the Krebs cycle.

2.4.1.3. Rhizobia and Nodulation:

Many fungicides show some degree of toxicity towards rhizobia and/or nodulation. Toxicity is often associated more with some strains of rhizobia than others. Afifi et al. (1969) found that at the lowest concentration (0.03 ppm) of captan, thiram, ceresan, dichlone and methyl arsenic sulphide (Rhizoctole) were toxic to some but not all rhizobia strains, whereas in the range of 300 to 30,000 ppm most strains were inhibited. According to Daitloff (1970), the in vitro toxicity of fungicides to rhizobia followed the order ceresan thiram captan chloranil.

In in vitro experiment with 33 fungicides and 21 strains of *R. leguminosarum* showed (Kecskes, 1970) that captan, thiram, dichlone and chloronil were moderately inhibitory whereas ceresan and MADD were strongly inhibitory. Gillberg (1971) found that *R. meliloti* strains generally tolerated higher rates of captan and a mixture of benzimidazole and carbamyl acid in vitro than did strains of *R. trifolii* and *R. leguminosarum*.

Investigations with thiram and its degradation products NaDDC, showed that some rhizobia were sensitive but the degree of sensitivity depended upon the strains of *Rhizobium* spp., and pH (Sud and Gupta, 1972). Using purest grade unformulated fungicides, Fisher (1976) showed little effect on the growth of *R. trifolii* in agar
containing thiram at highest concentration. Staphorst and Strijdom (1976) investigated the in vitro effects of 13 fungicides on rhizobia strains capable of nodulating *Vigna unguiculata*. Thiram was included among the most toxic group.

Thiram was found to inhibit *R. leguminosarum* but had no effect on nodulation of vetch in sand culture or in various soils under green house and light chamber conditions (Kecskes and Vincent, 1969a,b). Further inconsistencies between in vivo and in vitro experiment were demonstrated by Staphorst and Strijdom (1976). Thiram was found most toxic in vitro but had no effect on nodulation or dry mass of entire plants.

Van Assche (1974) showed that thiram had a growth stimulating effect on lettuce. Govindraju *et al.* (1975) showed that maneb and thiram at normal rates of application inhibited nitrogen fixation in the field. Only maneb inhibited the cell free nitrogenase. Using sterilized and non-sterilized soil, Fisher (1976) found that roots drenched with thiram and other fungicides at 25 and 50 Kg ha⁻¹ in each case, the dry weight and total nitrogen content of *R. trifolii* inoculated white clover plants were not affected. Thiram at the rate of 50 Kg ha⁻¹ increased N₂-fixation of excised nodules significantly. Application of thiram to bean seeds (*Phaseolus vulgaris*) inoculated with a thiram resistant strains of *Rhizobium* significantly increased dry weight and nitrogen content of plants (Linda and Alexander, 1981). Cossemans and Van Assche (1981) reported that some of the products, especially with dithiocarbamate structure, have the
capacity to promote growth directly. Thiram (0.2 and 0.45 %) treated seeds had no significant adverse effects either on the seed germination or on the nodulation. Grain yield was increased from 15.75 qtl ha\(^{-1}\) (control) to 17.33 qtl ha\(^{-1}\) (thiram, 0.2%) and 21.34 qtl ha\(^{-1}\) (0.45 %) (Sundaresh and Hiremath, 1982).

2.4.2. Effect of Dithiocarbamate on Nitrification:

Nitrification, the microbial oxidation of ammonia to nitrite and nitrate is carried out mainly by the nitrifying bacteria i.e., *Nitrosomonas* and *Nitrobacter*. Nitrification is one of the most sensitive of the microbial transformation to pesticidal residues (Kreutzer, 1963; Cullimore, 1971). The temporary suppression of the nitrification of ammonium-N is desirable in as much as the nitrogen is not lost from the plant rooting zone by leaching or by denitrification. But elimination of nitrifiers in treated soils may cause root injuries by ammonium accumulations or nitrogen deficiency disease of sugarcane (Dubey, 1970).

The effect of four dithiocarbamate on nitrification of ammonium sulphate were studied by percolation technique (Jaques *et al.*, 1959). Urethane (1.6 x 10\(^{-2}\) M Kg\(^{-1}\) soil) inhibited nitrification for 8 days whereas mane (2.1 x 10\(^{-4}\)M) and zeneb (2.1 x 10\(^{-4}\)M) for 25 and 8 days, respectively. Ferbam (3.5 x 10\(^{-4}\) M Kg\(^{-1}\) soil) inhibited nitrification for 28 days. These four dithiocarbamates caused a lag of 150 days for nitrification of ammonium sulphate when added into percolation units at a concentration of 2.1 x 10\(^{-3}\) Kg\(^{-1}\) soil.
Audus (1970) concluded that strong suppression of nitrification by dithiocarbamates followed by slow recovery, was in the following order (recovery time in days in parenthesis: nebam (60), thiram (60), ferbam (28), maneb (25) and zineb (17). Thiram (0.5 - 5 Kg ha\(^{-1}\)) either stimulated or had no effect on nitrification in grass soil. At higher rates, however, thiram (10 and 25 Kg ha\(^{-1}\)) inhibited the process of nitrification (Wainwright and Pugh, 1973). In an unspecified soil type, Wainwright and Pugh (1974) found that thiram (6.7 Kg ha\(^{-1}\)) inhibited nitrification markedly. Maneb and zineb applied repeatedly to soil resulted in decreased nitrification and nitrogen mineralization.

Agnihotri (1974) observed that different concentrations of TMTD (375, 187.5 and 93.7 ppm) impaired the process of nitrification while ammonification remained unaffected, as there was no considerable accumulation of ammonium nitrogen in fungicide treated soil. The amount of available phosphorous also increased following fungicide application. Benomyl (1.5 to 30 Kg ha\(^{-1}\)) in a humus sand decreased nitrification after 4 weeks incubation (Hofer et al., 1971) whereas in sandy soils receiving the equivalent of 1.0, 2.5 and 10 Kg ha\(^{-1}\). van Fassen (1974) found no effect for the first six weeks incubation but then significantly more nitrogen was found in treated soil than in untreated soil. In mixed cultures of *Nitrosomonas* sp. and *Nitrobacter* sp. however, van Fassen (1974) found that 20 ppm of benomyl inhibited oxidation of NO\(_2\) to NO\(_3\) and that 200 ppm of benomyl delayed the oxidation of NH\(_4^+\) and NO\(_2\).
as well as the oxidation of $\text{NO}_2$ to $\text{NO}_3$. Ramakrishna et al. (1979) showed inhibitory effect of benomyl (1000 ppm) on nitrification in flooded soil but benomyl at 10 ppm had little effect.

2.4.3. Effect on Ammonification:

In an acid lateritic clay (pH 5.0) and alluvial soil (pH 7.7) maneb and anilazine (24 Kg ha$^{-1}$) had no persistent effect on ammonification, but at 96 Kg ha$^{-1}$ inhibition occurred and no synergistic effect was observed (Dubey and Rodriguez, 1970). Thiram (0.5-25 ha$^{-1}$) applied to soil (pH 6.7; organic carbon 6.9%) increased ammonification (Wainwright and Pugh, 1973). Ammonification was also increased in soil (pH 5.3) treated with benomyl, captan and thiram (Wainwright and Pugh, 1974). Ammonium nitrogen increased in thiram (97.5, 187.5 and 37.5 ppm) treated soils as compared to control upto 6 weeks with the maximum increase registered at highest fungicide concentration (Agnihotri, 1974).

2.5. Soil Enzymes:

Soil enzymatic investigations have been increasingly employed in soil evaluation. Soil enzymes are known to be an index of soil biological activity and agricultural productivity. A direct relationship between soil fertility and enzymatic activity could be utilized for practical purposes (Burns, 1978; Qiu et al., 1981). A good correlation
between soil enzyme activity and microbial number (Skujins, 1967; Rodriquiz-Kabana and Truelove, 1970) and possible relationship between the soil microorganisms, exogenous substrates and extracellular enzymes in soil has been suggested (Burns, 1982).

In addition to the fundamental properties of enzymes in soil, the data from enzyme assays have been interpreted periodically as: guide to soil productivity (Kiss et al., 1978); an indirect measure of microbial biomass (Ladd, 1978; Oades and Jenkinson, 1979; Jenkinson et al., 1979; Domsch et al., 1979); a consequence of rhizosphere effect (Speir et al., 1981); an indication of the soil's potential for degrading both naturally occurring organic matter (Spalding, 1980) and xenobiotic compounds (Burns and Edwards, 1980) and a pointer towards any harmful side effects of pesticides (Lethbridge et al., 1981) on the microbiota.

Application of pesticides to soil under laboratory conditions significantly decreased soil microbial population and reduced activity of several enzymes such as phosphatase, saccharase, B-glucosidase and urease (Voets et al., 1974; Lethbridge and Burns, 1976; Cervelli et al., 1976). Maneb and Captan (10 μg g⁻¹ soil) inhibited amidase activity by three per cent (Frankenberger and Tabatabai, 1981).

The advantage of soil enzymatic investigations lies in the fact that they inform about the biochemical productivity of the soil microorganisms (Schinner et al., 1980).
Nutrients and other compounds are released through enzymatic activities and in this way, enzymatic reactions effect plant growth (Nielsen and Eiland, 1980).

Dehydrogenase activity was reported to be a good measure of total microbiological activity in soil (Casida, 1977). It was related to nitrogen fixation (Dorosinskii et al., 1966; Gupta et al., 1970) and interpreted as indirect measure of microbial biomass (Ladd, 1978).

Phosphatases are involved in the hydrolysis of phosphate ester bonds. But addition of phosphatases to soil was found ineffective in hydrolyzing phosphorus compounds (Jackman and Black, 1952). Khan (1970) reported increase in phosphatase activity by addition of fertilizer phosphorus in soil. Laugesen and Mikkelsen (1973) and Rolstone et al. (1975) showed inhibitory effect on the addition of inorganic phosphorus on the production and secretion of phosphatases by plants. However, the importance of phosphatases in the mineralization of organic phosphorus compounds to inorganic phosphorus which is available to plants is well known (Bowman and Cole, 1978; Rolstone et al., 1975). Biodegradation rates of 22 organic soils (Histosol) incubated for 39 days at 21°C were significantly correlated with acid phosphatase activity (Mathur and Levesque, 1980).

Amylase, cellulase and invertase are responsible for plant litter decomposition in soil (Pancholy and Rice, 1973) and were related to soil respiration (Rose, 1973). The enzyme peroxidase is important because of its role in
2.6. **Cellulase(s):**

Current forecasts for world energy sources coupled with the rapid utilization of non-renewable oil supplies and chronic food shortages, have lead to search for alternative energy and food sources. One of the processes being looked into for meeting the food and fuel shortages is the biodegradation of cellulose to glucose. The two main requirements here are: a reactive cellulose and active enzyme (cellulases) preparation. Considerable efforts have been devoted to increase the yield of cellulolytic enzymes both by optimizing cultural conditions and by searching for new mutants (Mandels, 1975).

It has now been clearly established from fractionation studies (Mandels and Reese, 1964; Li et al., 1965; Selby and Maitland, 1967; Bergham and Pettersson, 1973; Ghai, 1978), that at least three different types of enzymes are involved in the breakdown of native cellulose to simple sugars. The enzymes in cellulase complex consists of: C₁ enzyme attacking native cellulose; one or more Cₓ enzymes splitting the soluble chains and finally a B-glucosidase converting celllobiose to glucose.

Although effect of pesticides on the production of enzyme cellulases by *Trichoderma reesei* QM-9414 has not been reported by other workers, but considerable details are known regarding the optimal growth conditions for the production of maximal amount of cellulase(s) (Bailley et al.,
Some of the factors affecting cellulase production are mentioned in Table 3 given below:

Table 3: Factors affecting production of cellulase(s)

<table>
<thead>
<tr>
<th>Factors</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>5. Effect of sugars</td>
<td>Mandels et al., 1962; Nisizawa et al., 1972.</td>
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</table>

2.7. Development of resistance in Rhizobia:

In agriculture, it is important that efficient N₂-
fixing strains of rhizobia should be resistant to adverse environmental conditions. It is also desirable that adapted strains should not lose their efficiency or be modified in other ways which might reflect on the efficiency of N\textsubscript{2}-fixation.

Studies on the natural resistance and sensitivity of rhizobia to pesticides (Afifi et al., 1969; Sud and Gupta, 1972; Mackenzie and MacRae, 1972) have been made. The toxicity of several fungicides such as thiram, carboxin, captan and PCNB (Curley and Burton, 1975); phygon and spergon (Odeyami and Alexander, 1977) resulted in different levels of resistance depending upon the species, and even on the strains within the same \textit{Rhizobium} species.

Gupta and Kleczkowska (1962) observed that bacteriophage resistant mutants of \textit{R. trifolii} were unable to fix nitrogen. Schwinghaner (1964) showed that following the development of resistance in \textit{R. leguminosarum}, \textit{R. trifolii} and \textit{R. meliloti} to viomycin and neomycin, the normal symbiotic relationship with legumes was lost whereas no loss of effectiveness was observed in streptomycin resistant strains. Kaszubiak (1968a,b) studied the development of resistance against rhizobia to compound like afalon, aretite and Liro-betrax. Gillberg (1971) found that dinoseb and MCPA gave rise to rhizobia mutants, whose ability to infect legumes was not affected and that in MCPA induced mutants, nitrogen fixing ability was improved. Some of the mutants withstood higher than normal rates of the two herbicides, which might
be of use in combating possible harmful affects of herbicides on wild type strains in soils regularly treated with herbicides. Odeyami and Alexander (1977) studied the use of fungicides resistant rhizobia for legume inoculation. The fungicides resistant rhizobia retained their symbiotic effectiveness and survived fungicide treatments which were lethal to non-resistant rhizobia. Gupta and Shirkot (1981a,b) suggested that development of resistance to thiram and sevin in slow-growing rhizobia was in some way related to total lipids.

2.8. Microbial degradation of Dithiocarbamates:

An important part of soil microorganisms and pesticide interaction involves the rate of decomposition of a pesticide. The pesticides can be degraded by adapting the microorganisms. Microorganisms are important in the degradation of pesticides and precisely they are known as biological incinerators (Alexander, 1969). Microbial degradation accounts for much of the loss of the chemicals from soil (Kaufman and Kearney, 1970; Kaufman, 1974). While many organisms capable of degrading specific pesticides have been isolated and characterized, some chemicals failed to support microbial growth or enrichment during the degradation processes and others appeared persistent or otherwise resistant to microbial degradation. Hence the concepts of microbial fallability (Alexander, 1965; Alexander and Lustigman, 1966) and cometabolism (Horvath and Alexander, 1970; Horvath, 1970a,b) were introduced.
The mechanism by which soil microorganisms develop the capacity to degrade pesticides have been discussed adequately (Audus, 1960; Loss, 1969; Kaufman and Kearney, 1970). The principal biochemical reactions association with the microbial metabolism of pesticides include—alkylation, dealkylation, amide or ester hydrolysis, dehalogenation, dehydrogenation, dehydrohalogenation, oxidation, and conjugate formation (Kaufman, 1974).

The work of numerous investigators (Rich and Horsfall, 1950; Ludwig and Thorne, 1953; Ludwig et al., 1954; Richardson, 1954; Richardson and Munnecke, 1964; Vonk and Kaars Sijpesteijn, 1970, 1971; Marshal, 1977) contributed to the information on the chemical degradation of dithiocarbamates into various organic compounds while degradation by microorganism has been observed by few workers (Cox et al., 1951; Sisler and Cox, 1951, 1954; Weed et al., 1953). The information about the conjugate formation has also been reported (Kaar Sijpesteijn et al., 1962; Kaars Sijpesteijn and van der Kerk, 1965). Microbial involvement in the biodegradation of metham was not significant but chemical decomposition into methyl isothiocyanate, the primary toxicant was reported (Lloyd, 1962; Turner et al., 1962 and Turner and Corden, 1963).

2,8.1. Degradation of Thiram:

Thiram was introduced as field fungicide by Tisdale and William (1934). Few studies have been made
on the fate and persistence of thiram in the soil (Richardson, 1954; Kaars Sijpesteijn and Vonk, 1970; Munnecke, 1972), and its biodegradation by comparing persistence in sterilized and non-sterilized soil (Raghu et al., 1975).

Earlier investigations on the behaviour of thiram in soil were done using bioassay procedures (Richardson, 1954; Munnecke and Mickail, 1967). While these studies revealed the residual toxicity of thiram and/or its degradation products to the test organisms, information is lacking on the actual fate of the fungicide. Richardson (1954) indicated that the protection to plants lasted longer than did the fungicide. Munnecke and Mickail (1967) observed that soil treated with thiram retained the fungitoxicity for longer period than the fungicide.

Sisler and Cox (1951) observed the evolution of carbon disulphide by Fusarium spores treated with thiram. Thiram was shown to be reduced to dimethyldithiocarbamate by the spores of Glomerella cingulata (Richardson and Thorne, 1961). Rumen microflora degraded thiram to carbon disulphide and probably to hydrogen sulphide and dimethylamine (Robins and Kastelic, 1961).

Maeda and Tonomura (1968) isolated a pseudomonad capable of utilizing thiram as a source of carbon, nitrogen and sulphur. Degradation of thiram with this organism in the culture medium revealed dithiocarbamate (DDC), dimethylamine (DMA), formaldehyde, elemental sulphur and methionine as degradation products. Kaars Sijpesteijn
and Vonk (1970) suggested that thiram could be degraded to dimethylamine and carbon disulphide. Sud and Gupta (1972) reported the reduction of thiram to dimethyle dithiocarbamate by rhizobia. The formation of dimethylamine in thiram treated soils (Ayanaba et al., 1973 and Raghu et al., 1975), and in isolated microbial culture (Maeda and Tonomura, 1968 and Raghu et al., 1975) was demonstrated. Raghu et al. (1974,1975) reported the evolution of carbon disulphide in thiram treated unsterilized soil but not in sterilized soil. The isolate, a Pseudomonas sp. (Raghu et al., 1975) was not capable of utilizing thiram as sole source of carbon and metabolized thiram through cometabolism unlike the isolate of Maeda and Tonomura (1968). Carbon disulphide production from thiram in culture medium by Pseudomonas sp. was reported (Raghu et al., 1975). A possible degradation pathway for thiram in soil has been proposed by Raghu et al. (1975) based on their results and that of others.

2.9. Microbial decontamination of soil from pesticides:

Although pesticides are known to be degraded by different organisms, but the use of microorganisms for the decontamination of pollutants in the environment is a recently acknowledged feasibility. The potential problems involved with the use of microbial inocula in the environment have been discussed in reference to oil clean up (Cobet et al., 1973; Zobell, 1973). The use of microbial
preparations for the clean up of oil spills has been reported (Miget, 1973; Berner et al., 1975). Several oil degrading microbial preparations have been reported to be commercially available (Atlas and Bartha, 1973).

Only in the past decade have investigators shown that soil undesirably contaminated with a pesticide could possibly be decontaminated by inoculation with specifically adapted microorganisms. The idea, perhaps, was first embodied by Audus (1950) who showed that bacteria adapted to utilize the herbicide 2,4-D could accelerate its disappearance from soil when applied as inoculum. MacRae and Alexander (1965) made an attempt to protect the seeds of alfalfa from the herbicide by inoculating the heavy suspension of Flavobacterium sp. capable of utilizing this herbicide. All these studies were done under laboratory condition.

Kearney et al. (1969) showed accelerated DDT disappearance from flooded soil inoculated with DDT acclimated Enterobacter aerogenes. Clark and Wright (1970a,b) reported decreased phytotoxicity in IPC fortified soil inoculated with IPC utilizing Enterobacter sp. McClure (1972) performed most successful experiment on decontamination. Several phenylcarbamate herbicides were added to nonsterilized soil plots under greenhouse conditions at the rates up to 15 Kg ha\(^{-1}\). When a mixed bacterial culture capable of utilizing IPC as sole source of carbon was applied to soils, final plant yield were increased over
uninoculated controls. Another point of practical significance was that the functional activity of the acclimated cells in non-sterilized soil decreased exponentially, losing all effectiveness in 2 to 5 months. Daughton and Hsieh (1977) reported accelerated degradation of parathion in flooded and non-flooded soils by inoculation with parathion acclimated bacterial culture.